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(54) Title of the Invention: METHOD OF MANUFACTURING TEA CATECHINS

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### Specification

1. Title of the Invention:

METHOD OF MANUFACTURING TEA CATECHINS

2. Claims for the Patent:

(1) A method of manufacturing tea catechins characterized by comprising:

a first extraction process of extracting water-soluble components from tea leaves;

an adsorption process of adsorbing the water-soluble components to a chromatography column packed with a synthetic adsorbent or a gel filtration agent by charging the components to this chromatography column;

an elution process of eluting each of the adsorbed components with an aqueous solution containing about 40% to 100% of one of methanol, ethanol, and acetone or a mixture thereof; and

a second extraction process of transferring the above obtained eluate to one of ethyl acetate, methyl isobutyl ketone,

and diethyl ether or a mixture thereof and then evaporating organic components.

(2) A method of manufacturing tea catechins characterized by comprising:

a first extraction process of extracting water-soluble components from tea leaves;

a second extraction process of transferring the extract liquid to one of ethyl acetate, methyl isobutyl ketone, and diethyl ether or a mixture thereof and then evaporating organic components;

an adsorption process of adsorbing the extract components to a chromatography column packed with a synthetic adsorbent or a gel filtration agent by charging the components to this chromatography column; and

an elution process of eluting each of the above adsorbed components with an aqueous solution containing about 40% to 100% of one of methanol, ethanol, and acetone or a mixture thereof.

(3) The method of manufacturing tea catechins according to claim 1 or 2, characterized in that the tea catechins are a mixture containing four main components of epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate.

(4) The method of manufacturing tea catechins according to claim 1 or 2, characterized in that the synthetic adsorbent has the main component of styrene divinylbenzene or methacrylic acid ester.

(5) The method of manufacturing tea catechins according to claim 1 or 2, characterized in that the gel filtration agent is an

adsorbent with the main component of a dextran derivative or a hydrophilic vinyl polymer.

### 3. Detailed Description of the Invention:

#### [Industrial Application Field]

The present invention relates to a method of manufacturing tea catechins, and more specifically, relates to a method that can manufacture tea catechins of high purity at a low cost using tea leaves as a raw material.

#### [Conventional Art]

Tea catechins contained in tea leaves in a large amount are polyphenol compounds and a kind of flavanol. Main catechins contained in tea leaves are four types consisting of epicatechin, epigallocatechin, and gallic acid esters thereof, i.e., epicatechin gallate and epigallocatechin gallate. These tea catechins are known to have antioxidative activity, antibacterial activity, and other strong physiological activities.

In order to manufacture these tea catechins of high purity, elimination of impurities such as caffeine, brown pigments, amino acids, saccharides, steroids, and catechin oxides that coexist with catechins is indispensable.

Conventionally, a liquid-liquid extraction method is known as technique for collecting tea catechins of high purity from tea leaves. In this method, tea catechins extracted from tea leaves with warm water, hot water, or the like are transferred to another organic solvent.

For example, Yoshida (Chagyo Kenkyu Hokoku (Tea Research Journal), 1959 April, No. 13, page 4) describes a method of extracting a polyphenol mixture by extracting green tea with water, washing the extract with chloroform, and then further extracting with ethyl acetate.

In addition, in Japanese Patent Publication No. 1-44234, a natural antioxidative agent is manufactured by a similar method to the above.

In addition, in Japanese Patent Laid-Open No. 64-9922, a tea extract liquid is washed with hexane and chloroform, and then a target substance is extracted with ethyl acetate.

These methods utilize the characteristics that tea catechins are hydrophilic and also have high affinity to hydrophobic organic solvents, unlike saccharides, free amino acids, and so on. That is, tea catechins are selectively extracted and collected using these both solvents. However, the extraction requires a large amount of each type of solvents, and it takes a long time to obtain tea catechins of high purity. Thus, there are disadvantages in the manufacturing cost.

On the other hand, in a reference example described in Japanese Patent Laid-Open No. 63-135484, liquid-liquid extraction of a tea extract liquid is conducted using water and soybean oil, and only fat-soluble components are dissolved in soybean oil and removed. The aqueous phase is collected to obtain target tea catechins. In the thus collected components by this method, large amounts of saccharides and amino acids coexist with tea catechins. Consequently, the method has a

disadvantage that the purity of tea catechins is low as a matter of course.

The present inventors have developed adsorption/separation methods for collecting tea catechins of high purity by selectively adsorbing tea catechins to an adsorbent and eluting the tea catechins with an organic solvent (Japanese Patent Laid-Open Nos. 1-175978 and 1-135463). In these methods, a tea extract is injected into a chromatography column packed with a synthetic adsorbent, and impurities are previously removed by sequentially eluting them by changing concentrations of water and the organic solvent. The remaining tea catechins can be recovered at a high concentration.

However, the subsequent researches have revealed that tea catechins manufactured by the method of selective adsorption using such a packing agent contain brown materials that cause pigmentation. The brown materials are presumed to be oxidative polymerization products of tea catechins. Furthermore, these brown materials do not have antioxidative activity and other physiological activities and therefore are required to be removed for further improving the purity of tea catechins.

#### [Problems to be Solved by the Invention]

The present invention has been made in view of the above-mentioned problems, and an object of the present invention is to provide a method of manufacturing tea catechins having high purity and a high antioxidative activity or the like at a low cost by removing catechin oxidative polymerization products from

tea catechins obtained by an adsorption/separation method described above.

[Means for Solving the Problems]

That is, a first aspect of the invention is a method of manufacturing tea catechins characterized by including a first extraction process of extracting water-soluble components from tea leaves, an adsorption process of adsorbing the water-soluble components to a chromatography column packed with a synthetic adsorbent or a gel filtration agent by charging the components to this chromatography column, an elution process of eluting each of the adsorbed components with an aqueous solution containing about 40% to 100% of one of methanol, ethanol, and acetone or a mixture thereof, and a second extraction process of transferring the above obtained eluate to one of ethyl acetate, methyl isobutyl ketone, and diethyl ether or a mixture thereof and then evaporating organic components.

Furthermore, the extraction process for transferring to an organic solvent and the adsorption process by passing-through a chromatography column can be conducted in reverse order.

That is, a second aspect of the invention is a method of manufacturing tea catechins characterized by including a first extraction process of extracting water-soluble components from tea leaves, a second extraction process of transferring the extract liquid to one of ethyl acetate, methyl isobutyl ketone, and diethyl ether or a mixture thereof and then evaporating organic components, an adsorption process of adsorbing the extract components to a chromatography column packed with a

synthetic adsorbent or a gel filtration agent by charging the components to this chromatography column, and an elution process of eluting the above adsorbed component with an aqueous solution containing about 40% to 100% of one of methanol, ethanol, and acetone or a mixture thereof.

Furthermore, the aforementioned tea catechins are desirably a mixture containing four main components of epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate.

Furthermore, the aforementioned synthetic adsorbent is desirably an adsorbent with the main component of styrene divinylbenzene or methacrylic acid ester.

Furthermore, the aforementioned gel filtration agent is desirably an adsorbent with the main component of a dextran derivative or a hydrophilic vinyl polymer.

The present invention will now be more specifically described.

Figure 1 schematically shows processes of a method of manufacturing tea catechins according to the first aspect of the present invention.

The present inventors have combined a selective adsorption process using a conventional adsorbent and a liquid-liquid extraction process, and have investigated this method in order to obtain a white material by further improving the purity of tea catechins, compared to that of tea catechins obtained by conventional methods, by removing pigments such as the aforementioned brown materials.

Tea as a raw material of the present invention may be in any form such as raw leaves, unfermented tea, semi-fermented tea, or fermented tea. First, in the first extraction process, tea is extracted, for example, with hot water of 80°C or higher for about 5 to 10 minutes to give water-soluble components. In a case using a hydrous organic solvent such as methanol, in general, the extraction is conducted at a low temperature of about 60°C for a longer period of time.

These water-soluble components or tea extract liquid is concentrated and then injected into a chromatography column packed with an adsorbent for adsorbing various components to this adsorbent (adsorption process).

The above-mentioned adsorbent may be a synthetic adsorbent or a gel filtration agent. Examples of the synthetic adsorbent include usual synthetic adsorption resins such as methacrylic acid ester adsorbents, e.g., HP-1MG and HP-2MG (Mitsubishi Chemical Industries) and XAD-7 and XAD-8 (Organo); and styrene divinylbenzene adsorbents, e.g., S-761 (Duolite). Examples of the gel filtration agent include resins for gel filtration such as adsorbents with the main component of a dextran derivative or a hydrophilic vinyl polymer, e.g., Sephadex LH-20 (Pharmacia) and Toyopearl HW-40 (Tosoh).

Here, the aforementioned injection separates saccharides, metals, and protein from the chromatography column as an aqueous phase.

Then, the components remaining in the chromatography column is eluted using a hydrophilic organic solvent, for example, an



eluant containing about 40% to 80% of one of methanol, ethanol, and acetone or a mixture thereof (elution process).

The solvent for the elution is preferably the aforementioned hydrophilic organic solvent, and a concentration of 40% to 80% is effective for eluting tea catechins. When the concentration is 40% or less, the elution delays, and when the concentration is 80% or more, the effect does not increase with the concentration. Thus, both are uneconomical. A concentration of about 60% is preferred.

The solvent used for eluting tea catechins is concentrated once to remove the organic solvent and then supplied to the subsequent process (second extraction process). In a case that the organic solvent is not removed, the extraction efficiency in the following process decreases and the amount of the solvent used in the subsequent process increases. Thus, it is uneconomical.

Then, extract components are transferred from the thus solvent-removed liquid to an organic solvent (second extraction process).

The organic solvent is desired to be less dissolved in water, and the boiling point thereof is required not too high because of the necessity from removing the solvent. Therefore, the organic solvent is required to dissolve tea catechins to some extent but not to dissolve the brown materials at all. Examples of the solvent that can satisfy the above requirements are preferably ethyl acetate, methyl isobutyl ketone, or diethyl ether. In particular, ethyl acetate is desirable.

On the other hand, ethers other than diethyl ether, for example, isopropyl ether, benzene, chloroform, petroleum ether, and hexane, are unsuitable because of a low solubility of tea catechins. In addition, alcohols such as n-butanol also dissolve brown materials and are hence unsuitable.

In the second extraction process, catechin oxidative polymerization products are dissolved in the aqueous phase, and tea catechins are transferred to the organic solvent. Consequently, both are separated from each other.

In general, the organic solvent containing tea catechins is evaporated under reduced pressure, and the tea catechins are lyophilized or spray-dried into a powder. This powder is processed into various product forms.

Tea catechins of high purity can be manufactured by employing a two-stage processing by sequentially conducting a selective adsorption process using a packing agent and a liquid-liquid extraction process, as described above.

#### [Operation]

In the method of manufacturing tea catechins according to the present invention, tea catechins are adsorbed and isolated by treating a tea extract liquid by column chromatography and, in the next process, liquid-liquid extraction is conducted. Consequently, brown materials that are inseparable conventionally can be removed, and it is not required to use a large amount of a solvent. Therefore, tea catechins of high purity can be manufactured at a low cost.

[Examples]

The present invention will now be described with reference to Examples.

(Example 1)

One kilogram of green tea (Sencha) was extracted with 10 L of hot water for 10 minutes and squeezed. The resulting liquid was concentrated under reduced pressure and centrifuged to give 2 L of a tea extract liquid.

All this tea extract liquid was injected into a chromatography column packed with about 700 mL of a synthetic adsorbent (Diaion HP-2MG) with the main component of methacrylic acid ester, and then eluted with 1.7 L of a 70% ethanol solution.

This eluate is analyzed by high-performance liquid chromatography. The conditions for the high-performance liquid chromatography are as follows:

Chromatography column: CAPCELL PAK C18 (Shiseido)  
4.6 mm diameter × 250 mm length

Eluant for elution:

Eluant A: methanol/water/phosphoric acid  
(22%/78%/0.1%)

Eluant B: 100% methanol

Gradient:

0 to 15 min, eluant B: 0%

15 to 35 min, eluant B: from 0% to 20%

35 to 50 min, eluant B: from 20% to 100%

(Two-step gradient method, that is, first, eluant A is used for elution, and then the amount of an eluant is linearly increased from 0% to 20% during from 15 to 35 minutes after the injection

of a sample and from 20% to 100% during from 35 to 55 minutes after the injection.)

Flow rate: 1 mL/min  
Sample amount: 5  $\mu$ L  
Detector: ultraviolet absorptiometer  
wavelength: 0 to 22 min, UV: 280 nm  
22 to 50 min, UV 350 nm

Figure 2 shows the results of the aforementioned high-performance liquid chromatography analysis. As shown in the figure, tea catechins (four types: 1, 2, 4, and 5) with a purity of about 68% were obtained. The eluate containing this extract was concentrated under reduced pressure for removing ethanol to give about 500 mL of a liquid.

This liquid was treated with the same type of ethyl acetate three times. The ethyl acetate layers were combined and concentrated under reduced pressure, and lyophilized according to a usual method to give about 80 g of a white solid.

This powder was analyzed by high-performance liquid chromatography to confirm, as shown in Figure 3, that the peaks of brown materials disappeared and that the purity of tea catechins was about 85%.

#### (Application Example)

Tea catechins after the column fractionation and tea catechins after the ethyl acetate purification in Example 1 were each dissolved in a small amount of ethanol and investigated for the antioxidative activity thereof using linoleic acid. This

antioxidative activity was determined by the peroxide value (POV).

That is, each 10 mL of linoleic acid containing no tea catechins and containing 100 ppm of tea catechins was put in a 100-mL beaker and left in a thermostatic chamber at 40°C. The peroxide values (POV) were measured utilizing an iodo-starch reaction according to a usual method.

Figure 4 shows the resulting antioxidative activity curves. As shown in the figure, it was confirmed that the antioxidative activity of the purified product was higher than that of the column fractionation product.

#### (Comparative Example 1)

Extraction by a conventionally known method was conducted for comparison with the present invention.

1.5 liters of a tea extract prepared as in Example 1 was washed with the same amount of chloroform and then treated with 1.5 L of ethyl acetate three times (4.5 L in total) to transfer the extract components.

The ethyl acetate layers were combined, and the solvent was evaporated by concentration under reduced pressure. The residue was lyophilized to give 104 g of tea catechins. The purity of the tea catechins was 75%. Thus, the method of the present invention was superior to the conventional method in the amount of solvent used and the purity.

(Example 2)

A method similar to the method of Example 1 was performed. However, the organic solvent for purification was methyl isobutyl ketone or diethyl ether instead of ethyl acetate.

The results were that the recovery amounts of tea catechins were 75 g and 65 g, respectively, and the purity was 83% and 79%, respectively.

(Example 3)

A method similar to the method of Example 1 was performed. However, the column packing agent was LH-20 (Sephadex), HW-40 (Toyopearl), or S-761 (Duolite).

The results are shown in the table of Figure 5 together with the results in Example 1. There is substantially no difference due to the resin in the performance under this condition, except for S-761.

(Example 4)

Two liters of a tea extract liquid was manufactured by a method similar to that in Example 1 and was extracted with the same volume of ethyl acetate three times.

The ethyl acetate layers (about 6 L) were combined and concentrated under reduced pressure to evaporate ethyl acetate to give about 120 g of a solid. The solid was dissolved in water to give 500 mL of a solution. All this solution was injected into a chromatography column packed with about 175 mL of the same synthetic adsorbent (HP-2MG) as that in Example 1, and then eluted with 450 mL of a 70% ethanol solution.

This eluate was lyophilized similarly as in Example 1 to give about 80 g of a white solid.

Therefore, by changing the procedure of the manufacturing, namely, when the process is changed such that water-soluble components extracted from tea leaves are first transferred to an organic solvent, this organic solvent is then removed, and the water-soluble components are dissolving in water again and applied to a chromatography column, the amount of the adsorbent may be small and the loading amount can be increased, though a large amount of an organic solvent is used for the transferring.

In addition, the amount and the purity of the obtained product were the same as those in Example 1.

#### [Advantages of the Invention]

Thus, according to the present invention, tea catechins having a high antioxidative activity and other effective physiological activities can be manufactured inexpensively and at high purity, which cannot be achieved by conventional methods, by two-stage processing of a chromatography column adsorption/separation process and a liquid-liquid extraction process.

#### 4. Brief Description of the Drawings:

Figure 1 is an explanatory diagram schematically showing processes of purifying tea catechins according to the present invention;

Figure 2 is an explanatory diagram showing a chromatogram in adsorption/separation;

Figure 3 is an explanatory diagram showing a chromatogram in liquid-liquid extraction after the adsorption/separation;

Figure 4 is a graph showing the antioxidative activity of tea catechins manufactured by the method according to the present invention against linoleic acid; and

Figure 5 is a table showing the recovery amount and the purity of tea catechins.



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得た。これに水を加えて溶解させ、500ミリリットルとし、実施例1と同じ合成吸着剤 (HP-2MG) 約175ミリリットルを充填したクロマトカラムに全量注入したのち、70%エタノール450ミリリットルで溶離した。

この液を実施例1と同様に凍結乾燥して白色固形物約80gを得た。

したがって、製造法の手順を変更すると、つまり茶葉から抽出した水溶性成分をまず有機溶媒に懸濁し、この有機溶媒を除去後、再度水に溶解し、クロマトカラムに通すという工程を取ると、粗液のための有機溶媒量は多量に使用するが、吸着剤の量はすくなく済み、その負荷量を上げることができるという効果がある。

なお、得られた製品の量および純度は実施例1で実施した結果と同じである。

#### 【発明の効果】

以上のように本発明によれば、クロマトカラム吸着分離工程および液液抽出工程の二段処理に

より、高い抗酸化能その他有用な生理活性機能を有する茶カテキン類を従来にない高純度で安価に製造することが可能となった。

#### 4 図面の簡単な説明

第1図は本発明による茶カテキン類の新製方法の工程の概略を示す説明図。

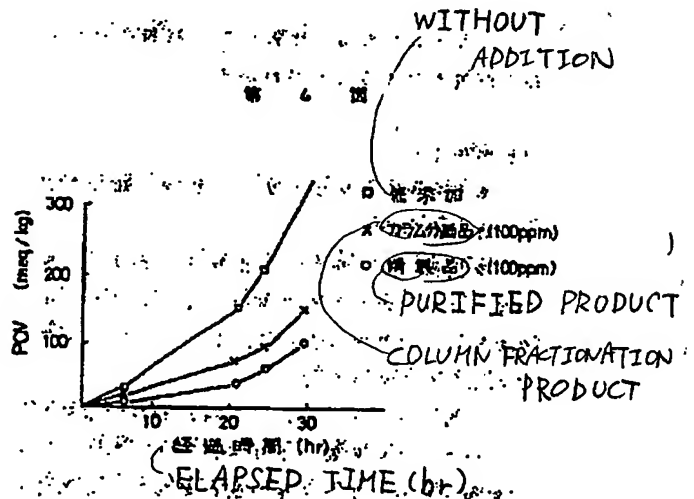
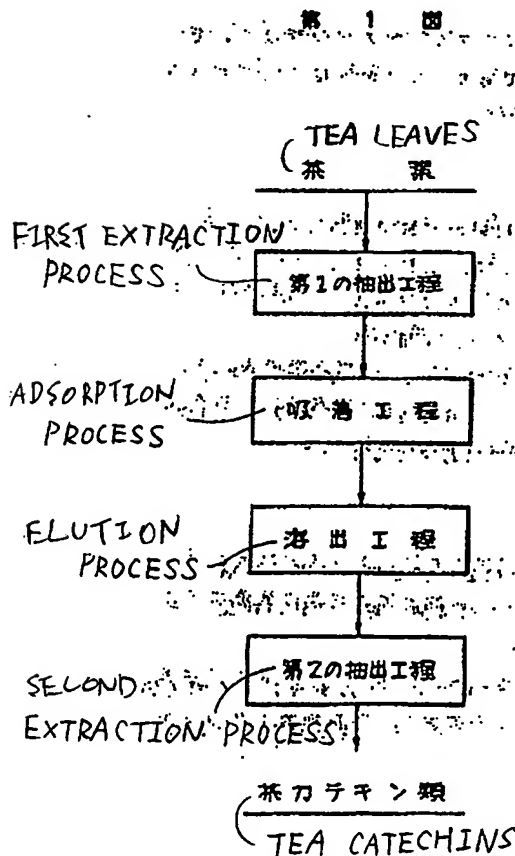
第2図は吸着分離によるクロマトグラムを示す説明図。

第3図は吸着分離ののち液液抽出によるクロマトグラムの説明図。

第4図は本発明の方法により製造された茶カテキン類のリノール酸に対する抗酸化能を示すグラフ。

第5図は茶カテキン回収率および純度を示す表である。

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第 5 図

RECOVERY AMOUNT AND PURITY OF TEA CATECHINS

茶カテキン類の回収率および純度

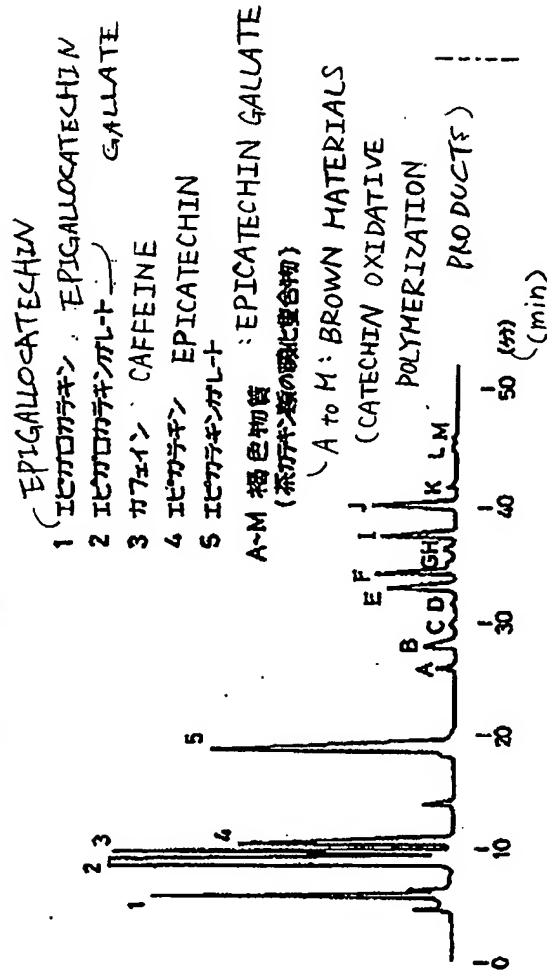
	HP2MG	LH20	HW40	S751
回収量 (gr)	80	85	82	62
純度 (%)	85	87	87	76

PURITY (%)

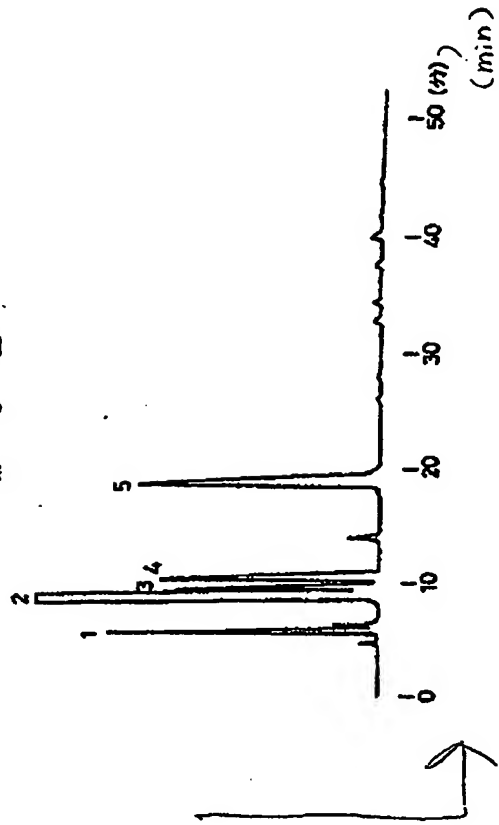
RECOVERY AMOUNT (gr)

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第 2 図



第 3 図



第 1 頁の続き

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Figure 1

- #1 TEA LEAVES
- #2 FIRST EXTRACTION PROCESS
- #3 ADSORPTION PROCESS
- #4 ELUTION PROCESS
- #5 SECOND EXTRACTION PROCESS
- #6 TEA CATECHINS

Figure 4

- #1 ELAPSED TIME (hr)
- #2 WITHOUT ADDITION
- #3 COLUMN FRACTIONATION PRODUCT (100 ppm)
- #4 PURIFIED PRODUCT (100 ppm)

Figure 5

- #1 RECOVERY AMOUNT AND PURITY OF TEA CATECHINS
- #2 RECOVERY AMOUNT (gr)
- #3 PURITY (%)

Figure 2

- #1 (min)
- #2 1: EPIGALLOCATECHIN
- #3 2: EPIGALLOCATECHIN GALLATE
- #4 3: CAFFEINE
- #5 4: EPICATECHIN
- #6 5: EPICATECHIN GALLATE
- #7 A to M: BROWN MATERIALS (CATECHIN OXIDATIVE POLYMERIZATION PRODUCTS)

Figure 3

#1 (min)